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SHORT COMMUNICATION

Optimal ultrasonic extraction condition and determination of polysaccharides in *Tilia amurensis* flowers

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Abstract: Seven extracting temperatures (25, 35, 45, 55, 65, 75, 85ºC) and extracting time (30, 40, 50, 60, 70, 80, 90 min) were designed for selecting the optimal extracting time and extracting temperature for ultrasonic extraction of *T. amurensis* flowers. Polysaccharides from *T. amurensis* flowers were isolated and determined by spectrophotometry. Results show that the optimal ultrasonic temperature was 75°C and extracting time 52 min. The content of polysaccharides in *T. amurensis* flowers measured by anthrone−H_2SO_4 colorimetry under 580 nm, was 9.74% with 0.47% of relative standard deviation (RSD, n=3). This study demonstrated that ultrasonic extraction method was simple, and the color of the treated samples was stable in 4 h. The average recovery value for the polysaccharides measured was 99.48%±1.01%, with 0.112% of RSD (n=3).

Keywords: best extracting condition; polysaccharides; *Tilia amurensis* flowers; ultrasonic extraction

Introduction

Tilia amurensis is a national secondary protection species and has high economic value in China (Mu et al. 2004). Current studies on *T. amurensis* mainly focused on the breeding and planting (Liu et al. 1998; Zhang et al. 2002; Zhang et al. 1998; Lv et al. 2001; Guo et al. 2001; Sun et al. 2003), three dimensional ultrastructure of wood (Peng et al. 1990). Flowers of *T. amurensis* are used as the medicinal part in Chinese traditional medicine, with sweating and antipyresis effects. In addition, in Russian folk, the flowers of *T. amurensis* were used to treat high blood pressure, cardiovascular disease and mood disorder during menopause. However, so far, no available information has been found on chemical compositions of *T. amurensis* flowers, especially on polysaccharides in *T. amurensis*. Ultrasonic extraction technology has been widely applied in the extraction of the plant compositions (Li et al. 2006). In the present study, we use ultrasonic extraction method to extract polysaccharides in *T. amurensis* flowers. The aim of this study was to determine the best extracting condition for the extraction of polysaccharides in *T. amurensis* flowers.

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Materials and methods

Materials

T. amurensis flowers were collected from the campus in Northeast Forestry University, Harbin, China in June 2005. All extractions were carried out by ultrasonic wave technology. Spectrophotometer was provided by Jiangsu Ronghua Instrument Ltd. Electronic scale (ALC-210) and electrothermal constant temperature blast drier were provided by Tianjin Test Instruments Ltd. High Speed Centrifuge (Anke TLD-40B) was provided by Shanghai Instrument Ltd. HH-2 Digital Contant Temperature Water-bath, SHB-3 Circulation Water Vacuum Pump, and Super Constant Temperature Implement were also used in this study. Anthrone, H_2SO_4, glucose, acetone, aether and ethanol (AR) were provided by Tianjin Chemical Reagent Co., Inc.

Sample preparation

The factors affecting ultrasonic extraction effective compositions in plants were analyzed under specific condition (Chen et al. 2007), including extracting solvent, ultrasonic frequency, extracting time, extracting temperature and the ratio of water to

materials. In this study we chose extracting temperature and extracting time as two affecting factors for ultrasonic extracting *T. amurensis* flowers. Seven extracting temperatures and extracting time were designed (Table 1). The best sample preparation method of polysaccharides content in *T. amurensis* flowers was determined according to polysaccharides content with evaluation indicator.

One gram of flower powder of *T. amurensis,* measured precisely, was put into 5-ml volumetric flask and added 3-ml distilled water. The test was repeated in triplicate. The ultrasonic extraction in the corresponding time was under the frequency of 40 kHz and the power of 100 W, and the extraction was repeated in triplicate. Then it was isovolumetric to the standard scale in 25-ml volumetric flask, after cooling to room temperature. A 1-ml supernatant was centrifuged at 12,000 rpm for 10 min to determine the extracting yield of polysaccharides.

Table 1. Experiment conditions and results of uniform design

Experimental Number	Extracting temperature(°C) X_1	Extracting time (min) X_2	Polysaccharides (mg/g)
1	25	70	8.7674
2	35	40	8.4448
3	45	90	9.7892
4	55	60	8.7069
5	65	30	9.6346
6	75	80	10.7303
7	85	50	10.4681

Uniform design experiment

Using extracting temperature (X_1) and extracting time (X_2) for independent variables, and polysaccharides extracting yield for causal variable, thus equation of the optimal regression were established by uniform design software as follows:

$$Y=0.7810505+0.7264183X_1+0.8298412X_2-0.0074052X_1X_2-0.0026145X_2^2$$

Confection of standard solution

Glucose standard sample (1 g) was dried at 105°C to constant weight; after then it was put into 100-ml volumetric flask, isovolumetric with distilled water to the standard scale. One milliliter standard solution was added to 100-ml volumetric flask, and was isovolumetric with distilled water to confect 100 g/mL standard solution. We added 1, 2 3 4, 5, 7, and 9 mL of standard solution separately into 10-ml volumetric flask, and then added distilled water to the standard scale to confect a series of standard solution (Fu et al. 2002).

Confection of anthrone–H_2SO_4 solution

A total 0.2-g anthrone was dissolved in 100-ml H_2SO_4 (98%) solution, held in brown bottle at first, then mixed and shook up, and finally put in a fridge (confecting when using) (Li et al. 2002).

Preparation of standard curve

We added 1-ml glucose standard solution into the tubes with plug, shaking up quickly, then put the tubes into ice water, and added 4 ml 0.2% anthrone–H_2SO_4 and boiled in the boiling water for 7 min after shaking up, and took out the tubes to cool quickly to room temperature by tap water. Finally the determining was conducted under 580 nm after laying 10 min (Han et al. 2006). Using glucose concentration for absorbency to regressive treatment, we obtained the regression equation as:

$$A = 0.0085C + 0.0069, r = 0.9987 \quad (1)$$

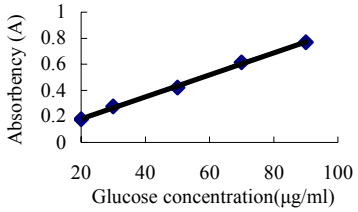

Fig. 1 Standard curve of glucose sample

Extraction and refination of polysacchrides

A total 100-g powder of *T. amurensis* flowers in dried constant weight was repeatedly extracted and defatted with petroleum ether (60–90°C) for 2 h twice, then extracted with 80% ethanol for 2 h twice for getting rid of monosaccharide oligosaccharide. The residue was extracted with five times distilled water in 90°C for 2 h twice. The extracts were mixed, decompressed, concentrated to 150 ml, and put in little active carbon to filtrate. The residue was put into 95% ethanol, and kept overnight. The precipitation with vacuum filtration was put in 100-ml water to dissolute, and repeated with ethanol once. The precipitation was washed with ethanol, acetone, aether repeatedly again, dried at 60°C, and finally the polysaccharides of *T. amurensis* flowers was obtained.

Conversion factors

Three micrograms of powder of *T. amurensis* flowers in dried constant weight were put into 20-ml volumetric flask, isovolumetric to the standard scale with distilled water (with the help of heating to dissolve polysaccharides), to obtain the polysaccharides stock solution. The absorbency (A) of polysaccharides stock solution was determined according to the above method and glucose concentration was obtained by the regressive equation. The conversion factor was determined by the following equation.

$$f = W/(C \times D) \quad (2)$$

where, f is the conversion factor, W the weight of polysaccharides (mg), C the glucose concentration in polysaccharides stock

solution in *T. amurensis* flowers (mg/ml), and *D* the diluent factor of polysaccharides.

Preparation of sample solution

One gram of flower powder was put into 50-ml 80% ethanol, and kept overnight, then filtrated. The residue was repeatedly extracted with 50-ml 80% ethanol for 1 h and heated-filtrated with filter paper. The sample was extracted twice. The residue was washed with hot ethanol and the solvent was air-dried. The residue with filter paper was put into flask and then extracted with 80-ml 100°C distilled water for 1 h, repeatedly extracted and heat-filtrated. The residue was washed using little distilled water, isovolumetric to the standard scale in a 250-ml volumetric flask, and then stored in a fridge at 4°C.

Polysaccharides content of sample

Sample preparation solution (1 mL) was taken for the determination of absorbency (A) according to the above method. Glucose concentration of the sample was obtained by regression equation, and polysaccharides content of *T. amurensis* flowers was calculated by the following equation:

$$\text{Polysaccharides content (\%)} = CDf/W \times 100 \quad (3)$$

where C is glucose concentration of sample preparation solution (μg/ml), D the diluent factor of polysaccharides, f the conversion factor, W the weight of polysaccharides (μg).

Recovery coefficient

We measured five shares of 1-g flower powder and prepared five sample solvents according to the above method. A sample solvent (1 mL) was put into the tubes with plug to determine the absorbency (A), and finally, we obtained the average polysaccharides content.

Stability test

We took out 1-mL sample stock solution to determine the absorbency (A) and repeated once at the interval of 1 h. After 4 h, the stability of the resultant color was observed.

Rate of recovery

One gram of flower powder in triplicate was put separately into 25-ml distilled water and added with 15-mg polysaccharides. The samples were extracted and determined under the above conditions.

Results and discussion

Optimal ultrasonic extraction condition

Stepwise regression model of each factor shows that the polysaccharides extracting yield was significantly affected by extracting temperature and extracting time. The optimal ultrasonic extraction parameters were determined as the ultrasonic temperature 75°C and extracting time 52 min according to comprehensive analysis of each test condition.

Conversion factor

The determined absorbency of polysaccharides was put in regression equation (Eq. 1) and the conversion factor calculated by Eq. 1 was f= 2.057.

Polysaccharides content and recovery rate

The absorbencies of three samples were put in regression equation and polysaccharides content was obtained according to equation 3. The average polysaccharides content was 9.74%, with RSD 0.47%.

Stability

The sample solution was stable basically in 4 h after athrone-H_2SO_4 chromogenic reaction (Table 2).

Table 2. Stability result of polysaccharides in different time

Time (h)	Absorbency (A)
0	0.488
1	0.487
2	0.487
3	0.486
4	0.485

Rate of recovery of treated sample

As shown in Table 3, the average recovery value for the polysaccharides measured was 99.48%±1.01%, with 0.112% of RSD (n=3).

Table 3. The result of recovery rate of treated sample

Sampling weight (g)	Original weight (mg)	Sampling weight (mg)	Absorbency (A)	Determination (mg)	Rate of Recovery (%)	Average rate of recovery (%)	RSD (%)
1.0003	82.095	15.3	0.489	97.217	98.83	99.48± 1.01	0.112
1.0001	81.49	15.1	0.485	96.412	98.81		
1.0001	82.902	15.6	0.496	98.63	100.81		

Discussion

The polysaccharide was extracted at high temperature, but extracting temperature was hard to control during the process of ultrasonic extraction. In this study we used a combined ultrasonic and super constant implement to control of stability of tempera-

ture. The anthrone-H_2SO4 reagent must be put through the tube wall slowly, or else parts of polysaccharides will be carbonized, thus leading to nigrifying the solution and affecting the accuracy of determination result.

The principle of determination of polysaccharides content of *T. amurensis* flowers is that the polysaccharides is hydrolyzed to the monosaccharide by the strong sulfuric acid and produced furol derivative rapidly. The synthesized colored compound can be shrunk with anthrone, and the coloration was linearly proportional to the monosaccharide. The structure and stability of the product of dehydration, the colors of monosaccharide and anthrone tautomers were different under the strong sulfuric acid. As the standard curve slopes of different monosaccharide are of marked difference, using glucose for standard curve will easily lead to system error. In this study, purificatory polysaccharides of *T. amurensis* flowers were used for conversion factor, thus system error was avoided to some extent by using glucose for standard curve.

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